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The Chemical Approach to Problems of Fermentation

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The Chemical Approach to Problems of Fermentation[1]

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The complexity of the chemical changes brought about by the action of microörganisms on carbohydrates and the profound effect of physical and chemical environment on the nature of these changes are pointed out. The importance of synergistic phenomena and their connection with industrial processes is discussed. The need for adequate quantitative methods of analysis is stressed, since the whole field of the biochemistry of fermentations is dependent on and limited by such methods. A chart is included which gives the interrelationships between substrates and products of fermentation.

Fermentation Terminology

BEFORE proceeding to the main topic under consideration it will be of interest to trace the evolution of the word "fermentation" and to point out the confusion caused by its use in several meanings. The word comes from the Latin, *fervere*, "to be boiling hot." It was used by Pasteur to designate microbiological action, especially upon saccharine materials, in which such quantities of gas were evolved as to produce an appearance of boiling or effervescence. The agencies bringing about this type of change were called "ferments." Later it was learned that dead cells as well as extracellular products were capable of bringing about fermentations in the original sense—that is, produced gas during their action. Hence the terms "organized" and "unorganized" ferments. The latter name then became synonymous with enzymes and the term "fermentation" came to include any enzymatic action whether or not gas is evolved. It is seen, then, that the phenomenon of fermentation was

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originally not synonymous with utilization. That is, the statement that an organism would not ferment a certain substrate simply meant that gas was not evolved, not that the material failed to serve as a metabolite. Later the statement became more inclusive and carried the connotation that the organism could not utilize the substrate—that is, was not capable of bringing about chemical transformations, leading to its utilization in the living processes of the cell. Hence the statements in the older and newer literature in regard to the ability or inability of an organism to ferment a given substrate may not have the same meaning.

In discussion involved with industrial fermentations the term "fermentation" refers to the microbiological transformations, predominately of the carbohydrates and their dissimilation products. The carbohydrates are emphasized, since these materials furnish the important sources of energy for the organisms and for the zymotechnical production of chemicals on a large scale.

From a chemical viewpoint zymology (fermentation) deals with catalysis (or rather autocatalysis) in heterogeneous system. Industrial catalyses and zymotechnical syntheses differ in that in the former case the catalyst, usually a simple type of chemical, is manufactured outside the reacting mixture and then added to the reactant or reactants under controlled conditions. In the latter processes the catalysts, the enzymes, are manufactured during the course of the reaction. This involves a knowledge of the nutrition and characteristics of the organism used and the conditions under which it will produce in the highest degree the particular catalysts desired. Different organisms bring about different chemical changes in the same medium and the chemism of a given organism varies in the same substrate if nutrients and physical chemical environment are altered.

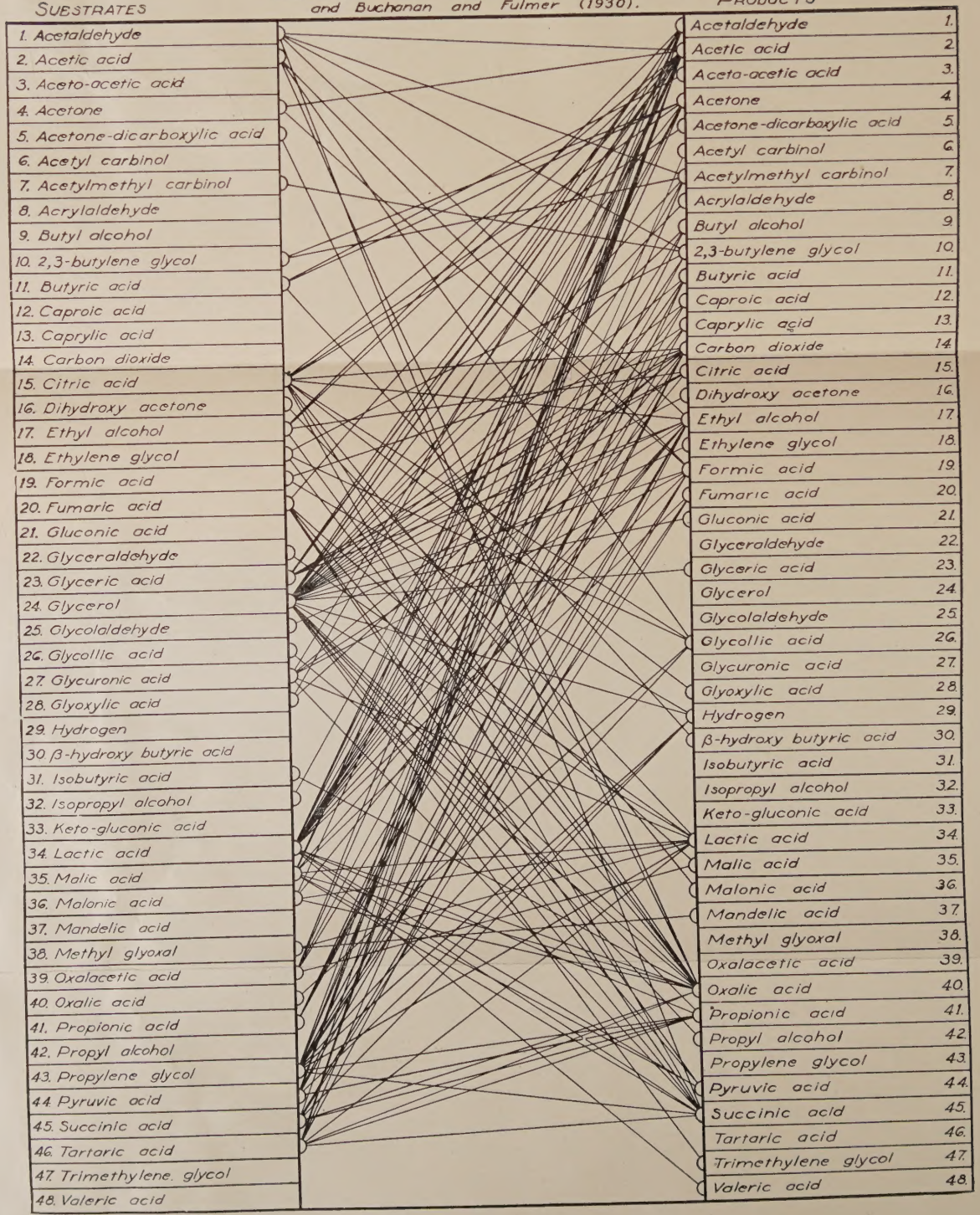
For example, molds commonly produce citric acid from carbohydrates, while bacteria do not. One set of products may predominate under anaerobic conditions, while others are in excess under aerobic conditions. The butanol and acetone fermentation industry depends for maximum yields upon the use of the appropriate organism under anaerobic conditions. Yeast produces only small quantities of glycerol relative to ethanol in the normal or slightly acid medium, while in an alkaline medium or in the presence of sodium sulfite the relative yields of these compounds are reversed. These facts are basal to the commercial production of glycerol by fermentation. The chemical yields are likewise influenced by the temperature aside from the effect upon reaction rates.

Moreover, there are many instances known in which two different organisms growing together bring about chemical actions which neither alone could effect. This phenomenon is known as "synergism." Synergistic action is very complex in detail and a real understanding of the phenomena

CHART I.

The Fermentative Interrelationships of the Microbiological Dissimilation Products of the Carbohydrates [a]

Summarized from Fulmer and Werkman (1930) and Buchanan and Fulmer (1930).



involved awaits a great deal of research and the development of more adequate chemical technics. Evidently pure-culture industrial fermentations are not always the most desirable, and it is likely that some of the most striking advances in zymotechnology lie in the utilization of synergistic phenomena.

Zymotechnology differs in approach from industrial syntheses in that in the latter the catalyst brings about a practically complete final reaction without a complexity of intermediate products—that is, the action is drastic. In fermentation we are dealing with a series of graded products or stages in oxidation and reduction. The formation of the fermentation products is the process whereby the organism secures the energy for growth and maintenance. Evidently the organisms do not usually secure the maximum energy yield by a complete oxidation of the carbohydrates to carbon dioxide and water. It is owing to this fact that there is such a thing as the fermentation industry.

The various theories of the mechanism of fermentation attempting to rationalize the complex oxidation-reduction relationships of the many fermentation products have led to the development of a unique terminology involving such terms as "donators," "acceptors," "hydrogenations," "dehydrogenations," "hydrations," "dehydrases," "activators," "dislocation," "dismutation," "catalytic protoplasmic groups," "graded protoplasmic affinities," and an imposing array of enzymes involved in various details of microbiological dissimilation. In fact, the terminology threatens to become as imposing as that developed by Ehrlich and school with reference to immunology.

Chemicals Produced by Action of Microörganisms on Carbohydrates

The complexity of the chemical problems involved may be illustrated by a brief survey or summary of the chemicals reported as produced by the action of microörganisms upon the carbohydrates and upon the intermediates. In Table I are listed the products of the action of microörganisms on the hexoses and pentoses. It is of interest to note the similarity of the products from these monosaccharides, especially in view of the fact that the industrial fermentation of agricultural wastes involves a fermentation of the pentosans.

Chart I summarizes the fermentative interrelationships of the microbiological dissimilation products of the carbohydrates. These include the products identified as being produced by the action of bacteria, yeasts, or molds. The dissimilation products are arranged alphabetically in the left-hand column as substrates. In the right-hand column are the same materials classed as products. The lines connecting a member of the left-hand column with certain members of the right-hand column represent the products formed

by the action of bacteria, yeasts, or molds on the given substrate. Conversely the lines connecting a given product with given substrates show at a glance the different substrates from which the product may be formed by microbiological action.

Table I—Reported Products of Action of Microörganisms on Hexoses and Pentoses[a]

Acetaldehyde	+Fumaric acid
+Acetic acid	+Gluconic acid
+Acetone	Glycerol
+Acetylmethyl carbinol	Glycolic acid
+Butyl alcohol	+Hydrogen
+2,3-Butylene glycol	Ketogluconic acid
+Butyric acid	+Lactic acid
Caproic acid	Malic acid
Caprylic acid	Methylglyoxal
+Carbon dioxide	+Oxalic acid
+Citric acid	+Propionic acid
+Ethyl alcohol	Pyruvic acid
+Formic acid	+Succinic acid

[a] All the items in the table are produced from the hexoses and those marked + are likewise produced from the pentoses.

The alphabetical arrangement in the chart is wholly arbitrary. The ideal arrangement would be in the order of free energies of formation. However, such values are available for only a few of the chemicals listed. This at once emphasizes the need of the calculation of these values for all the compounds involved before these interrelationships can be interpreted on a strict basis. Other methods of arrangement might be on the basis of structure, intermediate products, or heats of combustion.

The chart immediately shows the complexity of the chemical problems involved. One of the first needs is the development of adequate quantitative methods of analysis of such mixtures. Many methods now used are qualitative or semiquantitative at best. Until such methods are developed, it is impossible to formulate a complete flow sheet for a given organism under given conditions. Moreover, adequate methods may show traces of compounds which are of fundamental importance in any complete theory of the mechanism of the fermentation involved.

It is evident that the fermentation products are not simply degraded products of the substrate, but that in many instances there occur synthetic reactions. Reference to the products from acetaldehyde shows this point in striking fashion. In fermentation processes the reagents (enzymes) are relatively mild agents, so that in effect the process from inception to completion—that is, to carbon dioxide and water—is magnified and spread over considerable periods of time. Thus, in microörganisms there are presented unique tools for the production of chemicals. Each item in the table and chart holds the possibility of commercial production by fermentation processes. It is evident that only a few of the possibilities have been exploited. The problem resolves itself into the bringing together of the right organism or organisms and the right medium under optimum conditions.

Recent Reviews on Fermentation

(1) Buchanan and Fulmer, "The Physiology and Biochemistry of Bacteria," Vols. I, II, III, Williams and Wilkins, 1928 to 1930.

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(4) Nord, "Mechanism of Enzyme Action and Associated Cell Phenomena," Williams and Wilkins, 1929.

(5) Schoen, "Le problème des fermentations, les faits et les hypothèses," Masson, Paris, 1926.

(6) Stephenson, "Bacterial Metabolism," Longmans, 1930.

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